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STEM-END ROT OF ORANGES AND FACTORS AFFECTING ITS CONTROL[1]

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INTRODUCTION

Stem-end rot of citrus fruit has received a great deal of critical study and has been the subject of many scientific and popular publications. In spite of the excellent work pertinent to its control, however, it remains a major cause of losses to the retailer and consumer as well as to the grower and shipper of citrus fruit from the southeastern part of the United States. In a study of storage diseases of grapefruit a few years ago, Brooks and McColloch (*4*)[3] found that 12 to 60 percent (average 30 percent) of the Florida fruit purchased on the market in Washington, D. C., developed stem-end rot if held in storage at 50° F. or higher for as long as 8 weeks.

In the present studies stem-end rot was found to be a serious cause of spoilage of Florida oranges purchased on the Washington market. When the fruit was held for a week at a temperature of 75° F. in an atmosphere having a high relative humidity, stem-end rot resulting from infection in the grove occurred on 7 to 47 percent (average 28 percent) and was the main cause of loss. It seems evident that this disease is the most important cause of loss in Florida oranges to the retailer and the consumer. Much of this loss may be due to failure to use such methods of control as are available, but comprehensive investigations by Winston (*13*) indicate that part of the trouble may be due to a lack of methods that are completely satisfactory.

As most of the previous studies of stem-end rot have been made on fruit infected in the grove, it was thought desirable in preliminary experiments to inoculate oranges that were known to be free from stem-end rot and then to disinfect them. The results of such experiments as well as of those on naturally infected oranges are reported herein.

MATERIAL AND METHODS

The earlier investigations were conducted at Beltsville, Md., on oranges purchased on the Washington market. The fruits were as fresh as could be found, but in many cases the buttons had lost considerable of their greenness and freshness. In most cases, California oranges were used since these were free from stem-end rot, but the results obtained were checked with Florida oranges both for disease control and for possible injury to the fruit. The later experiments,

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[3] Italic numbers in parentheses refer to Literature Cited, p. 380.

1940–42, were made at Orlando, Fla., on naturally inoculated fruit fresh from the tree.

Under each experimental condition at Beltsville, 18 or more California oranges were used, and the number of Florida fruits was 40 or more, often 100. In the experiments at Orlando 100 or more fruits were used under each condition.

In the inoculation experiments suspensions of *Diplodia* or *Phomopsis* spores were applied to the buttons with a medicine dropper, and the oranges were allowed to stand with the buttons up till the inoculum was nearly dry. Aside from the exceptions mentioned later (pp. 344 and 345), an effort was made to apply the inoculum to both the cut stem and the surrounding calyx. After inoculation the oranges were placed in a large metal cabinet where the temperature was held at about 75° F. and the relative humidity was near the saturation point. In the earlier experiments the fruit was kept in the cabinet throughout the test, but in the later ones it was moved at the end of 7 days to a room where the temperature was 70° and the relative humidity 85 to 90 percent.

In the experiments at Orlando, the oranges were taken as they came from the grove without artificial inoculation. They were carefully sorted, and after disinfection and other treatments were held in a basement room at a temperature of about 70° F. and a relative humidity of 80 to 90 percent.

In testing the effect of gases 5-gallon jars were used and a high humidity was maintained by the use of wet paper towels. When the jars were to remain filled with fruit for a considerable period, small openings were made near the bottom to prevent the accumulation of carbon dioxide. When high-humidity treatment was desired before disinfection, either 5-gallon jars or pony refrigerators were used.

OBTAINING AND GERMINATING SPORES OF THE CAUSAL ORGANISMS

Rapid decay of oranges starting at the buttons or stems may be caused by either of two fungi, *Diplodia natalensis* Pole-Evans [4] or *Phomopsis citri* Fawc.[5] In the course of the experiments a slow stem-end decay caused by a species of *Alternaria* was occasionally found on California fruit. A slow stem-end decay due to *Colletotrichum gloeosporioides* Penz. sometimes occurred on relatively green Florida oranges, especially those previously treated with borax. The present studies were confined largely to the first two organisms.

One of the great difficulties in laboratory studies with these two fungi is that they do not sporulate readily and consistently in culture. In efforts to obtain ready sporulation various types of culture media were tested. The best results with *Phomopsis* were obtained with sterilized snap beans in test tubes. Pods in which the beans had developed to considerable size seemed to be better than those that were less mature. Surplus water was usually drained from the test tubes at the time of inoculation.

Good spore production by *Diplodia* was obtained on seeds of wheat. The wheat was soaked overnight, the excess water was drained off, and the wheat was then sterilized in test tubes or as a shallow layer in

[4] Perfect stage, *Physalospora rhodina* (Cke.) Berk. and Cke.

[5] The perfect stage, *Diaporthe citri* Wolf, has been reduced by Wehmeyer (*12*) to synonymy with *D. medusaea* Nitschke.

small flasks. At 80° F. abundant production of hyaline single-celled spores was obtained in 12 to 20 days after inoculation. If used within 10 days, these spores produced rapid decay of oranges when applied to the buttons in a spore suspension. When the test-tube or flask cultures were held for much more than 5 weeks at a temperature of 80°, the spores failed to germinate in water, nutrient solutions, or nutrient agar. This was probably due to some sort of staling or aging, but dryness may also have been a factor since spores were found to be very sensitive to drying after they had issued from the pycnidia. Spores held moist for 15 minutes on a slide and then allowed to dry rapidly failed to germinate later.

Very few brown thick-walled two-celled spores were found in the inoculum obtained from the wheat cultures even several weeks after the spores had issued from the pycnidia. In some cases actively fruiting cultures were dug out of the flasks or test tubes, broken into small pieces, and allowed to dry slowly in closed Petri plates in the laboratory. In these, two-celled spores soon predominated and the remaining one-celled spores gave a very low percentage of germination. When filtered out of a spore suspension and allowed to dry on filter paper, the two-celled spores remained viable for several days under laboratory conditions.

The fact that the spores produced as described did not always prove viable led to germination studies with the different spore forms. These were carried out by placing the spores in water and in prune juice in the concavities of culture slides and exposing them to various storage conditions. In water the one-celled and the lighter colored two-celled *Diplodia* spores germinated freely, but the darker two-celled *Diplodia* spores and the *Phomopsis* spores often germinated very poorly. In prune juice, however, all spore forms germinated well.

Since, as discussed on page 373, ethylene treatments are known to increase stem-end rot, tests were made to determine whether any of this increase might be due to better spore germination. The results showed that exposure in an atmosphere containing 1 to 4,000 ethylene brought the germination of *Phomopsis* and two-celled *Diplodia* spores in water up to more than half that found in prune juice. Equally great stimulation, however, was obtained by placing two oranges or the rind of two oranges in a 5-gallon jar with the spore cultures. Evidence has been obtained recently (*10*) that oranges give off ethylene. It is possible that the stimulus from the presence of oranges was due to ethylene, although it is also possible that other gases given off by the oranges may have had some effect.

Vapors from various chemicals were also found to affect germination. Placing 2 cc. of ethyl acetate, 2 cc. of ethylene chlorhydrin, or a small quantity of menthol in a 5-gallon jar with the water cultures gave a decided increase in germination of two-celled *Diplodia* spores. On the other hand, the vapors given off by geraniol, clove oil, and alcohol had an inhibiting effect. Stimulation and inhibition of spore germination by volatile substances have been reported by others (*1*, *5*).

THE ROLE OF THE STEM BUTTONS

The stem buttons furnish the point of entry for the stem-end rot fungi (fig. 1) and also afford a protected place for the lodgment of spores.

The calyx lobes lie rather close on the surface of the rind, forming a projecting ledge under which spores and dirt can collect and be fairly well protected from wind and sun. Even after several hours of expo-

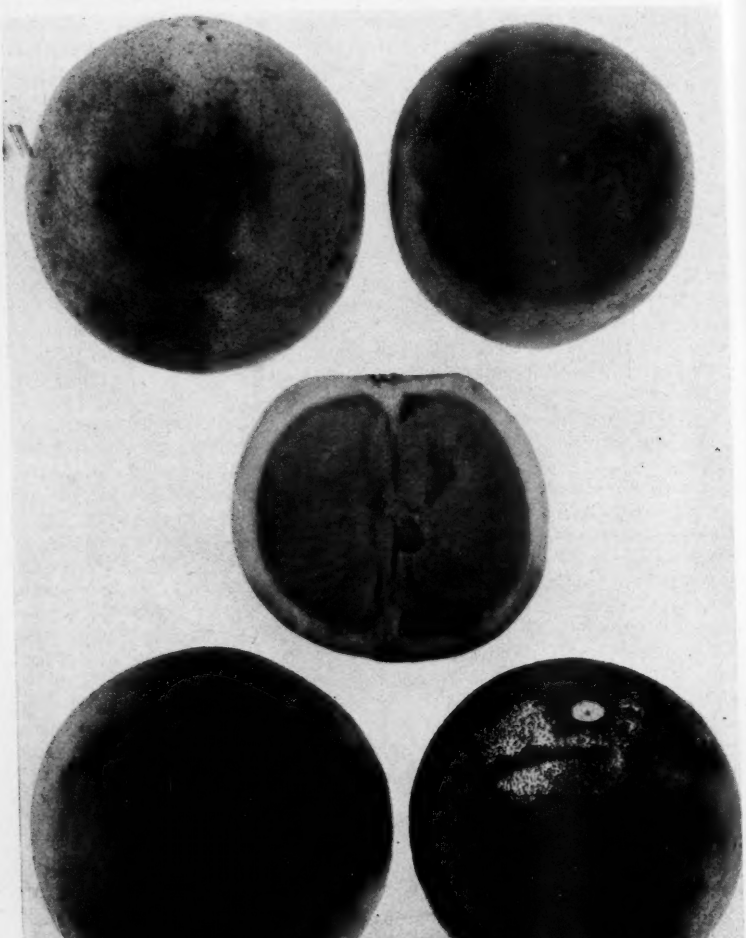

FIGURE 1.—Stem-end rot of oranges at various stages. Note the growth of the causal fungus on the exterior of the orange at lower right.

sure to bright sunshine the fruit surface beneath the calyx does not appear entirely dry when examined with a hand lens.

Stem-end rot can be almost entirely prevented by the early removal of the buttons, but no generally practicable method has been found

for doing this. Winston (*15*) recently showed that in grapefruit grown in certain sections of Florida losses from stem-end rot can be prevented by pulling the fruit and leaving the buttons behind instead of clipping as has been the standard practice. Clipping, however, is necessary for the orange crop because pulling may result in tears in the rind that furnish points of entrance for green and blue molds (*Penicillium digitatum* Sacc. and *P. italicum* Wehmer).

Although it is generally recognized that the stem-end rot fungi gain entrance to the fruits through the buttons, there has been some question whether they enter through the cut stems or through the calyx lobes. Tests were made to obtain evidence on this point.

In one set of experiments a mixture of beeswax, paraffin, and mineral oil was used in paste form to localize the infection. On part of the oranges the cut stems were coated and on others the entire buttons except the cut stems were coated before inoculating with *Phomopsis*. California-grown Washington Navels and Valencias were used in the tests. After 2 weeks at 75° F. 80 percent of the stem-inoculated Washington Navels and 69 percent of the calyx-inoculated ones showed decay; at that time 38 percent of the stem-inoculated Valencias and 13 percent of the calyx-inoculated ones showed decay. When the Valencias were held for an additional 2 weeks, the stem-inoculated fruit showing decay had increased to 92 percent and the calyx-inoculated to 35 percent.

Several tests were made to determine whether decay could be produced by applying spores to the scars left upon the removal of the buttons. The results were contradictory, the degree of infection probably varying inversely with the extent to which separation layers had formed beneath the buttons.

In May 1940 a test was made at Orlando to determine the path of natural infection in freshly harvested Valencia oranges. Merthiolate (sodium ethylmercuri-thiosalicylate) 1 to 1,000 in 50 percent alcohol was applied in one lot to the cut stems only and in another lot to the calyxes only. Part of the fruit was then given a 2-day ethylene treatment, and part was stored immediately. After 4 weeks at 70° F. 45 percent of the ethylene-treated fruit without merthiolate, 19 percent of that with merthiolate applied to the cut stems, and 3 percent of that with merthiolate applied to the calyxes had decay. In similar lots that were not treated with ethylene the percentages were 8, 1, and 0, respectively. In both cases the application to the calyx was more effective than that to the cut stem. However, much more merthiolate was required to cover the calyx than to cover the cut stem, and this quantitative difference may have been significant. In the ethylene-treated fruit most of the decay was due to *Diplodia*, and in the untreated fruit most of it was due to *Phomopsis*.

The results of the various experiments indicate that the stem-end rot fungi can make their start on either the stem or the calyx lobes and probably on any part of the button. These results are in agreement with data reported by Fawcett (*6*).

RELATIVE SPEED OF PENETRATION BY PHOMOPSIS AND DIPLODIA

Evidence as to the relative speed of penetration by the two organisms was obtained by the removal of the buttons or by the application

of a disinfectant at various periods after inoculation and also from records as to the time of appearance of decay on the control lots of fruit. In these experiments the inoculum was applied to all parts of the button. A brief statement of the results has already been published (*2*).

The effect of the time of removal of the buttons from Washington Navel oranges is shown in table 1, from which it is evident that *Diplodia* makes its way through the button in less than half the time it takes *Phomopsis*.

TABLE 1.—*Effect of time of removal of buttons from inoculated Washington Navel oranges upon the development of stem-end rot*

[*Phomopsis* data, from a single experiment; *Diplodia* data, average of 3 separate experiments]

Organism used	Period between inoculation and button removal	Fruit with stem-end rot—		
		1 week after inoculation	2 weeks after inoculation	4 weeks after inoculation
		Percent	*Percent*	*Percent*
Phomopsis	Check (not removed)	0	68	95
	3 days	0	0	0
	4 days	0	0	4
	6 days	0	5	17
Diplodia	Check (not removed)	79	97	
	1 day	7	12	
	2 days	8	15	
	3 days	43	52	

The effect of the time of borax application upon the development of stem-end rot is shown in table 2. It will be noted that treatment 4 days after inoculation was almost as effective against *Phomopsis* as treatment on the day of inoculation, but that treatments delayed 2 or 3 days were of little value against *Diplodia*. Winston (*14*) pointed out the importance of early disinfection in any attempt to control stem-end rot.

TABLE 2.—*Effect of the time of borax application on the development of stem-end rot in Washington Navel oranges*

Treatments with an 8-percent solution at 100° F. for 2 minutes; fruit held at 75°; A, average of 6 tests; B, average of 5 tests; C, average of 4 tests]

Lot and organism used	Time of borax application	Fruit with stem-end rot—		
		1 week after inoculation	2 weeks after inoculation	4 weeks after inoculation
		Percent	*Percent*	*Percent*
A (*Phomopsis*)	Check (untreated)	0	39	79
	Day of inoculation	0	9	14
	1 day after inoculation	0	3	21
	2 days after inoculation	0	4	15
	3 days after inoculation	0	7	16
	4 days after inoculation	0	11	24
B (*Diplodia*)	Check (untreated)	78	92	
	Day of inoculation	9	30	
	1 day after inoculation	21	33	
C (*Diplodia*)	Check (untreated)	71	98	
	1 day after inoculation	10	44	
	2 days after inoculation	55	84	
	3 days after inoculation	72	93	

It should be noted that in the check lots (table 2) *Diplodia* had produced more decay at the end of 1 and 2 weeks than *Phomopsis* at the end of 2 and 4 weeks, respectively. This contrast in the activity of the two organisms is further emphasized in the experiments reported in figure 2, where it is shown that *Diplodia* had caused as much wastage at the end of 4 and 7 days as *Phomopsis* at the end of 9 and 13 days, respectively. That this difference was not due to temperature is indicated by the fact that the tests were made at approximately the

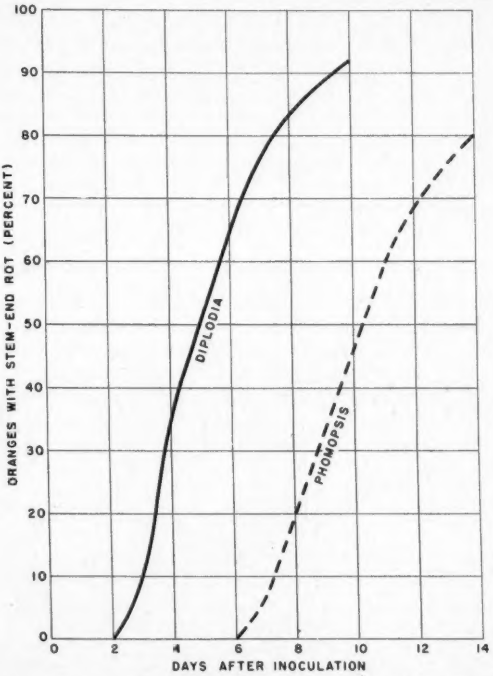

FIGURE 2.—Average rate of development of stem-end rot in Washington Navel oranges inoculated with *Diplodia* and *Phomopsis* and held at approximately 75° F. (Seven tests with each organism.)

optimum for *Phomopsis*, which is 65° to 75° F. (7), and below the optimum (86°) for *Diplodia*.

The various experiments indicate that under conditions favorable to both fungi *Phomopsis* would be so completely outdistanced by *Diplodia* that it would produce little decay.

In one set of inoculation experiments with *Diplodia* a comparison was made between inoculum in which about 99 percent of the spores were one-celled and inoculum in which about 90 percent of the spores were two-celled. Under conditions favorable for the germination of both spore forms (p. 365) there was no difference in the rate at which decay developed.

DISINFECTION EXPERIMENTS WITH HAND-INOCULATED FRUITS

When the present studies were begun it was thought that if fruit known to be free from stem-end rot was used and uniform methods of inoculation were employed the results with each disinfectant would be consistent. But such was not the case. A disinfectant that gave almost complete control in one instance sometimes failed almost entirely in another, and one that stood at the top of the list in one test sometimes fell to the bottom of the list in the next. It was not found advisable, therefore, to accept results without several repetitions.

No completely satisfactory explanation of the variability in results has been found. The degree to which the inoculum was allowed to dry before the fruit was stored and the period between the time of inoculation and the time of disinfection apparently had an important bearing on the results. Evidence was also obtained that a chemical which kills or weakens the buttons without killing all the spores may actually increase decay even though it is known to be a fairly good disinfectant. Since in previous studies (*13*, *14*) borax was found to be the best disinfectant for stem-end rot, it was used in all the present experiments for comparison with other materials tested.

FORMALDEHYDE TREATMENTS

A number of experiments were carried out with formaldehyde. Preliminary tests indicated that a solution containing 0.2 percent of formaldehyde was as strong as could be used without injury to the fruit, unless the treatment was to be followed immediately by washing, and that a solution containing 1.5 percent was as strong as could be used even when followed by washing. Even at these strengths formaldehyde sometimes caused a few red specks on some of the oranges.

TABLE 3.—*Stem-end rot of oranges treated with borax and formaldehyde 1 day after inoculation*

A, Average of 3 tests on Washington Navel and of 1 on Valencia oranges grown in California; B and C, average of 2 and 3 tests, respectively, on Washington Navel oranges grown in California]

Lot and organism used	Treatment	Fruit with stem-end rot — 1 week after inoculation	2 weeks after inoculation	4 weeks after inoculation
		Percent	*Percent*	*Percent*
A (*Phomopsis*)	Check (untreated)	----------	93	97
	8 percent borax at 110° F. for 2 minutes; not washed.	----------	27	38
	0.2 percent formaldehyde at 110° F. for 2 minutes; not washed.	----------	15	25
	4 percent borax + 0.2 percent formaldehyde at 100° F. for 2 minutes; not washed.	----------	11	23
B (*Diplodia*)	Check (untreated)	44	64	----------
	5 percent borax at 75° F. for 2 minutes; not washed.	10	13	----------
	0.2 percent formaldehyde at 75° F. for 2 minutes; not washed.	42	56	----------
C (*Diplodia*)	Check (untreated)	79	98	----------
	5 percent borax at 75° F. for 2 minutes; washed after 2 days.	25	31	----------
	1.5 percent formaldehyde at 75° F. for 2 minutes; washed immediately.	55	68	----------

The results of experiments with formaldehyde indicate that it might be of value in the control of decay by *Phomopsis*, but that it is of little or no value in the control of decay by *Diplodia* (table 3). It,

therefore, could not be expected to give satisfactory results with fruit that is likely to carry both fungi. Since there seemed to be no justification for the commercial use of the material, tests were not made to determine whether formaldehyde could be detected in the treated fruit.

CHLORINE TREATMENTS

Calcium and sodium hypochlorites were tested as disinfectants for stem-end rot of oranges. The results are shown in table 4. The treatments as carried out did not cause appreciable injury, but from the standpoint of stem-end rot control they must be regarded as almost complete failures.

TABLE 4.—*Stem-end rot of oranges treated with borax and with chlorine-containing compounds at 80° F. for 2 minutes 1 day after inoculation*

[A, 1 test on Washington Navel oranges; B, average of 3 tests on Washington Navel oranges; C, average of 1 test each on Washington Navel and Florida Pineapple oranges; D, test on Washington Navel oranges; E, average of 3 tests on Washington Navel oranges; F, average of 2 tests on Washington Navel oranges and one on Florida Pineapple oranges; G, average of 2 tests on Washington Navel oranges; Washington Naval oranges grown in California]

Lot and organism used	Treatment	Fruit with stem-end rot—		
		1 week after inoculation	2 weeks after inoculation	4 weeks after inoculation
		Percent	*Percent*	*Percent*
A (*Phomopsis*)	Check (untreated)	----------	58	100
	5 percent borax; not washed	----------	0	0
	Calcium hypochlorite (available chlorine 0.25 percent); not washed.	----------	24	76
B (*Diplodia*)	Check (untreated)	30	54	----------
	5 percent borax; not washed	7	12	----------
	Calcium hypochlorite (available chlorine 0.25 percent; not washed.	15	70	----------
C (*Diplodia*)	Check (untreated)	54	96	----------
	5 percent borax; washed after 3 days	26	46	----------
	Calcium hypochlorite (available chlorine 1.25 percent); washed immediately.	59	95	----------
D (*Phomopsis*)	Check (untreated)	----------	59	100
	5 percent borax; not washed	----------	0	0
	Sodium hypochlorite (available chlorine 0.07 percent); not washed.	----------	21	74
E (*Diplodia*)	Check (untreated)	3	31	----------
	5 percent borax; not washed	0	0	----------
	Sodium hypochlorite (available chlorine 0.07 percent); not washed.	2	20	----------
F (*Diplodia*)	Check (untreated)	71	83	----------
	5 percent borax; washed after 1 day	20	22	----------
	Sodium hypochlorite (available chlorine 0.14 percent); washed immediately.	61	81	----------
G (*Diplodia*)	Check (untreated)	66	85	----------
	5 percent borax; washed after 2 days	14	33	----------
	Sodium hypochlorite (available chlorine 0.35 percent); washed immediately.	47	64	----------

PHENATE TREATMENTS

In preliminary experiments two different phenate compounds were tested as orange disinfectants: Sodium ortho-phenylphenate and a trade product that is a mixture of sodium 2-chlorphenylphenate and sodium tetrachlorphenate. In the same concentration the two chemicals gave approximately the same control of stem-end rot. The mixed product was sometimes slightly more effective, but it was much more likely to cause specking and spotting of the oranges and it also had an undesirable effect upon flavor. On some oranges this could not be detected, but when high-quality fruit was used a definite

loss of character and flavor was evident a few days after treatment. No such unfavorable effect was observed with the sodium ortho-phenylphenate.

Van der Plank and Rattray (*11*) reported favorable results in the control of *Penicillium digitatum* (green mold) decay of citrus fruit with a dip in a 0.3- to 0.5-percent solution of sodium ortho-phenylphenate. Hwang and Klotz (*9*) obtained decided reduction in the spore germination of *P. digitatum* with a 5-minute exposure to a 0.15-percent solution of the same material.

Sodium ortho-phenylphenate is the only phenyl compound used in the experiments reported, and for the sake of brevity the material is sometimes referred to herein as phenate solution. In preliminary experiments it was not found advisable to use the phenate treatment without following it with washing. When not followed by washing, a 0.2-percent solution left spots on the oranges, yet it did not give satisfactory control of stem-end rot. All the treatments reported in table 5 were followed by washing, and none of them caused injury.

TABLE 5.—*Stem-end rot of oranges treated with borax and phenate at 80° F. for 2 or 3 minutes at different periods after inoculation with Diplodia and held the first week at 75° and the second at 70°*

[A, Florida Pineapple oranges; B to D, California Washington Navel oranges. Fruit in D exposed to ethylene 1 day before disinfecting]

Lot	Treatment	Fruit with stem-end rot—	
		1 week after inoculation	2 weeks after inoculation
		Percent	*Percent*
A	Check (untreated)	54	92
	5 percent borax 1 day after inoculation; washed after 3 days	45	67
	1.2 percent phenate 1 day after inoculation; washed immediately.	6	46
B	Check (untreated)	77	99
	5 percent borax 1 day after inoculation; washed after 3 days	23	47
	1 percent phenate 1 day after inoculation; washed immediately	30	54
	5 percent borax 2 days after inoculation; washed after 3 days	54	83
	1 percent phenate 2 days after inoculation; washed immediately	19	54
C	Check (untreated)	73	83
	5 percent borax day of inoculation; washed after 1 day	24	39
	1.2 percent phenate day of inoculation; washed immediately	21	54
	5 percent borax 1 day after inoculation; washed after 1 day	15	34
	1.2 percent phenate 1 day after inoculation; washed immediately.	7	34
	1.2 percent phenate 1 and 3 days after inoculation; washed immediately.	0	0
D	Check (untreated)	49	79
	5 percent borax 1 day after inoculation; washed after 1 day	19	53
	1.2 percent phenate 1 day after inoculation; washed immediately.	3	27

At the end of 1 week there was decidedly more decay of the borax-treated fruit than of the phenate-treated fruit in four out of six possible comparisons, but at the end of 2 weeks this difference had largely disappeared. The longer period of exposure in the case of borax may have influenced the results. It seems probable that the borax may not have been completely washed off after once becoming thoroughly dry and was therefore present to cause inhibition or killing at a later date. It should be noted that particularly good results were obtained in the one instance (table 5, lot C) in which the

phenate treatment was given at the end of 1 day and again at the end of 3 days. There was some indication that in advanced stages of infection phenate treatments were more effective than borax treatments (table 5, lots B and C).

EXPERIMENTS WITH NATURALLY INFECTED FRUIT

In April and May 1939, 1940, and 1941, and in the fall, winter, and spring of the 1941–42 season experiments were carried out at Orlando on naturally infected fruit. It was not possible to separate the decay caused by *Diplodia* from that caused by *Phomopsis* on the basis of general appearance, and, in order to be certain as to the prevailing organism and the relative resistance to control, large numbers of cultures were made in 1940, 1941, and 1942.

Both *Diplodia* and *Phomopsis* were found to be present in abundance in the spring of 1940 and in the fall and winter of 1941–42, but in the spring of 1941 all the decay was due to *Phomopsis*. The spring of 1941 was unusually cool, and this may account for the entire absence of *Diplodia* in the experimental fruit at that time.

ETHYLENE TREATMENTS

Ethylene treatments are considered an almost indispensable seasonal procedure in the preparation of citrus fruit for market, because of the unfavorable impression made by fruit that contains any green color in the rind. Early and midseason oranges do not lose all their green color by the time the fruit is ready for market. To some extent this is also true of the late-ripening varieties and in addition these often regreen before the end of the shipping season. In order to remove the green from the rind, ethylene treatments are given for 1, 2, and sometimes even for 3 and 4 days in rooms in which the temperature is held at 80° to 85° F. and the relative humidity at 87 to 92 percent.

It is well known that these ethylene treatments weaken the fruit and very greatly increase the later development of stem-end rot. When both organisms are present the decay that develops on ethylene-treated fruit is likely to be due almost entirely to *Diplodia*, whereas that which develops on untreated fruit is likely to be due to *Phomopsis* (*3*, *8*, *13*, *14*, *16*). The effect of ethylene treatment on decay is shown in figure 3, *A*, which gives the average of the results in eight separate experiments in the 1941–42 season. It will be noted that nearly all the increase from ethylene treatment was due to *Diplodia*.

The results naturally raise two questions: (1) What is there in the ethylene treatments that is so much more favorable to *Diplodia* than to *Phomopsis*? (2) Since *Diplodia* can produce decay in half the time required by *Phomopsis*, why does not *Diplodia* cause more decay than *Phomopsis* when the fruit is not treated with ethylene? There are at least three factors that may have a bearing on these questions. The first, naturally, is temperature. The temperature of the ethylene rooms is near the optimum for *Diplodia* and above the optimum for *Phomopsis* (p. 369). This would undoubtedly give *Diplodia* a decided advantage during the period of treatment and for a considerable time thereafter, but it can hardly account for the large increase in *Diplodia* rot even into the third and fourth week of holding.

Another factor to be considered is the aging and weakening effect of ethylene treatments on the buttons. This is undoubtedly favor-

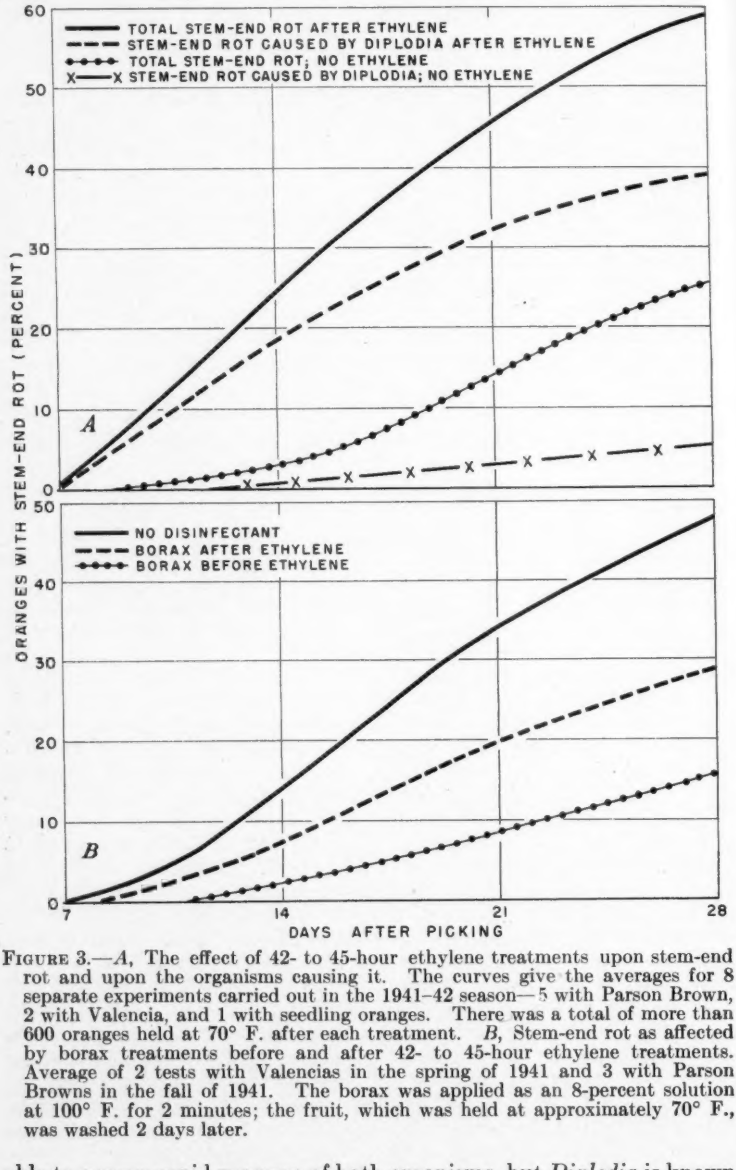

FIGURE 3.—*A*, The effect of 42- to 45-hour ethylene treatments upon stem-end rot and upon the organisms causing it. The curves give the averages for 8 separate experiments carried out in the 1941–42 season—5 with Parson Brown, 2 with Valencia, and 1 with seedling oranges. There was a total of more than 600 oranges held at 70° F. after each treatment. *B*, Stem-end rot as affected by borax treatments before and after 42- to 45-hour ethylene treatments. Average of 2 tests with Valencias in the spring of 1941 and 3 with Parson Browns in the fall of 1941. The borax was applied as an 8-percent solution at 100° F. for 2 minutes; the fruit, which was held at approximately 70° F., was washed 2 days later.

able to a more rapid progress of both organisms, but *Diplodia* is known to be less actively parasitic than *Phomopsis* and is probably more

favored by the aging and weakening of the buttons. It is believed that the effect of ethylene on the buttons is probably the most important factor in the increase in *Diplodia* decay.

A third factor is the effect of ethylene upon spore germination. As stated earlier, the fully mature two-celled spores of *Diplodia* do not germinate freely in water but germination may be stimulated by ethylene. Upon free exposure to the air the one-celled spores soon die or pass over into the two-celled form unless conditions are favorable for immediate germination. In showery weather the fruit might be expected to carry a liberal supply of freshly exuded one-celled *Diplodia* spores, but under average weather conditions it is likely that only the two-celled form would be present and that ethylene would help to start them into activity.

The results shown in figure 3, *A*, however, should not be taken as proof that the ethylene treatment has no effect upon *Phomopsis* decay. In experiments made in the spring of 1941 with fruit that carried no *Diplodia*, it was found that at the end of 2 weeks at 70° F. 21 percent of the ethylene-treated fruit on an average had decayed as compared with 14 percent of the untreated fruit. Apparently the usual dominance of *Diplodia* on ethylene-treated fruit is due not to any inhibition of *Phomopsis* but rather to the naturally more rapid growth of *Diplodia* and the creation of conditions favorable to its development.

FORMALDEHYDE, CHLORINE, AND SODIUM BISULFITE TREATMENTS

Various disinfectants have been tested on naturally infected fruit, but at strengths which caused no injury most of them gave little control of stem-end rot; in fact, stem-end rot was sometimes increased.

Formaldehyde was tested both as a gas and as a liquid. It usually gave reduction in stem-end rot, but never a satisfactory control even when used at strengths that caused injury to the rind.

Chlorine was used as a gas and in the form of sodium hypochlorite solutions. Oranges showed little injury even with extreme treatments, but stem-end rot was increased almost as often as it was decreased. Sodium bisulfite solutions were tested at various strengths but the result was usually an increase in stem-end rot. Increase in stem-end rot resulting from chlorine and sodium bisulfite treatments was apparently due to the chemicals causing greater injury to the buttons than to the fungi, the dead or weakened buttons thus furnishing favorable conditions for the rapid advance of the decay organisms.

BORAX AND SODIUM ORTHO-PHENYLPHENATE TREATMENTS

In the experiments with hand-inoculated fruit borax and sodium ortho-phenylphenate were the only materials tested that gave promise of having commercial value, and they were given many comparative tests on naturally infected oranges.

Effect of Time of Application

As pointed out previously (p. 372), with hand-inoculated oranges there was some indication that phenates were effective at a more advanced stage of infection than borax. The same was true with naturally infected fruit and was especially evident in treatments given before and after ethylene. Figure 3, *B*, presents a comparison

made between borax treatments before and after the fruit had passed through the coloring room. During the first 3 weeks of holding there was more than twice as much stem-end rot on the oranges treated after ethylene as on those treated before ethylene.

The importance of applying borax as soon after picking as possible and the benefits of applying it before ethylene treatment have been repeatedly emphasized by Winston (*13*, *15*, *16*) and others.

A similar series of experiments was made in which phenate was applied as a 1.2-percent solution at 100° F. for 2 minutes and the fruit was washed immediately. At the end of 2 weeks in three tests with Parson Browns, four with Valencias, and two with Jaffas an average of 2.1 percent of the oranges treated after ethylene had stem-end rot as compared with 3.3 percent of those treated before ethylene and 11.8 percent of the untreated ones. In contrast with the results with borax, the phenate treatments after ethylene made a somewhat better showing in decay control than the treatments before ethylene.

The contrast in the results with the two fungicides is probably due partly to the difference in methods of application. The borax was left on the fruit for 2 days and the phenate for only 2 minutes. It has been suggested that the fungi had penetrated too deep into the button tissue during the ethylene treatment to be reached by borax, and the curves of figure 3, *B*, seem to give some support to this idea; yet the failure to control with the after-ethylene treatment was nearly as great with the rots appearing at the end of 3 and 4 weeks as with those appearing at the end of 9 to 14 days. It seems probable that much of the advantage of applying borax before ethylene is due to the greater penetration of the chemical into both fungus and button tissue under the high temperature and high humidity of the ethylene room and the less complete removal in the later washing.

The results with phenate indicate that the decay organisms had not penetrated too deep to be reached by a disinfectant at least after 42 to 45 hours in the ethylene room. In fact, fruit on which the phenate treatment was delayed after removal from the ethylene room often showed less decay than fruit treated immediately after removal. An example of this is shown in table 6. In this case the results from disinfecting 1, 2, or 3 days after removal from the ethylene room were better than those from disinfecting immediately after removal.

TABLE 6.—*Stem-end rot of Parson Brown oranges held at 70° F. as affected by delays in and repetitions of phenate treatment after a 42-hour period in the ethylene room*

[Phenate applied as a 1.2-percent solution at 100° F. for 2 minutes; fruit washed immediately]

Lot	Treatment	Fruit with stem-end rot—		
		10 days after harvest	12 days after harvest	14 days after harvest
		Percent	*Percent*	*Percent*
A	Check (untreated)	1	8	20
B	Phenate immediately after removal from ethylene room	1	2	11
C	Phenate 1 day after removal from ethylene room	0	0	4
D	Phenate 2 days after removal from ethylene room	0	0	2
E	Phenate 3 days after removal from ethylene room	1	1	2
F	Phenate immediately and 1, 2, and 3 days after removal from ethylene room.	0	0	0

This apparent advantage from delaying the phenate treatments, however, is based on records of decay at a given number of days after harvest. If the table were changed to show the percentage of stem-end rot a given number of days after the disinfecting treatment the advantage of the delayed treatments would largely disappear. From the practical point of view, the important fact is that phenate treatments were fairly effective even when there was considerable delay in applying them. The phenate as applied apparently had an inhibiting rather than a killing effect. After 2 or more weeks at 70° F. the organisms on the treated fruit again became active. This was avoided by a daily repetition of the disinfecting treatment as shown in lot F (table 6), a method that of course would be impracticable under most commercial conditions.

Effectivenss of Various Treatments

The foregoing results make it evident that the data from ethylene-treated fruit and untreated fruit should be considered separately and also that phenate treatments after ethylene are likely to be of more practical value than phenate treatments before ethylene.

Table 7 gives further data on the relation of ethylene and phenate treatments to the development of stem-end rot. Phenate-treated oranges that had received ethylene had approximately the same amount of stem-end rot as the untreated fruit that had not received ethylene.

Table 7.—*Stem-end rot of oranges held at 70° F. as affected by treatment with phenate*

[Phenate applied as a 1.2-percent solution at 100° F. for 2 minutes 2 days after harvest; fruit washed immediately. Average of 5 separate experiments (2 with Valencia and 1 each with Parson Browns, seedlings, and Jaffas). Ethylene treatment for 42 to 45 hours]

Lot	Treatment	Fruit with stem-end rot—			
		9 days after harvest	14 days after harvest	17 days after harvest	21 days after harvest
		Percent	*Percent*	*Percent*	*Percent*
A	Ethylene treated, no disinfectant	3	12	29	45
B	Phenate after ethylene	1	3	8	15
C	No ethylene, no disinfectant	0	3	9	20
D	Phenate, no ethylene	0	0	0	4

With fruit that had not been in the coloring room the disinfecting treatment delayed the development of stem-end rot approximately 1 week and with fruit that had received ethylene there was a corresponding delay of approximately half a week. Any lowering of the temperature below 70° F. would increase the spread in time. Under the usual shipping conditions the lower temperatures probably would double these time periods.

The control obtained with phenate after ethylene was not as complete as that shown in figure 3, *B*, for borax before ethylene, but it was better than that obtained with borax after ethylene. The treatments reported in table 7 did not cause injury; yet it was found that a 1.2-percent solution would sometimes cause rind injury if the temperature rose much above 100° F. or if the treatment was not followed by thorough washing. It seemed evident that 100° should

be regarded as a maximum temperature for safe operation with this strength of solution.

Various modifications in the phenate applications were tested. At 120° F. injury was obtained with 0.8- and 0.5-percent solutions and the decay control was poorer than with a 1.2-percent solution at 100°. Increasing the temperature with a particular strength of solution increased injury more rapidly than decay control. The most satisfactory results were obtained at 100° or lower. Increasing the period of treatment seemed to be favorable to decay control without greatly increasing injury.

In view of these observations tests were made at lower temperatures and under conditions that seemed likely to extend the period that the disinfecting agent would be active. In some cases the phenate treatments were followed immediately by dipping in a water-wax emulsion and in others the phenate was added to the water phase of the wax emulsion. The results of these experiments are shown in table 8.

TABLE 8.—*Stem-end rot of oranges as affected by various 2-minute treatments*

All lots except B4 run in duplicate. A and B, Seedling oranges stored at 65° F.: A, 42-hour ethylene treatment after borax and before phenate; B, no ethylene. C, Valencia oranges stored at 80° F.: 1 and 2, no ethylene; 3 to 10, inclusive, ethylene; 4 and 5, treatments before ethylene; 6 to 10, treatments after ethylene]

Lot		Treatment	Fruit with stem-end rot—				
			11 days after harvest	14 days after harvest	17 days after harvest	21 days after harvest	28 days after harvest
			Percent	*Percent*	*Percent*	*Percent*	*Percent*
A	1	Check (no disinfectant)	5	24	44	----------	----------
	2	8 percent borax at 100° F.; washed after 2 days	2	4	10	----------	----------
	3	1.2 percent phenate at 100° F.; washed immediately	2	7	17	----------	----------
	4	1.2 percent phenate at 100° F.; then wax emulsion	0	3	18	----------	----------
	5	2.0 percent phenate at 80° F.; washed immediately	0	3	18	----------	----------
	6	2.0 percent phenate at 80° F.; then wax emulsion	0	6	16	----------	----------
B	1	Check (no disinfectant)	0	1	8	14	28
	2	1.2 percent phenate at 100° F.; washed immediately	0	0	1	1	6
	3	1.2 percent phenate in wax emulsion at 100° F	0	0	0	0	5
	4	1.2 percent phenate in wax emulsion at 100° F. (dipped only)	0	0	0	0	5
C	1	Check (no disinfectant)	1	4	9	16	----------
	2	1.2 percent phenate at 100° F.; washed immediately	1	1	1	4	----------
	3	Check (no disinfectant)	4	11	22	40	----------
	4	8 percent borax at 100° F.; washed after 2 days	1	2	4	7	----------
	5	1.2 percent phenate at 100° F.; washed immediately	1	4	12	29	----------
	6	1.2 percent phenate at 100° F.; washed immediately	0	0	3	18	----------
	7	1.2 percent phenate at 90° F.; washed immediately	0	0	3	18	----------
	8	1.2 percent phenate at 100° F.; then wax emulsion	0	1	4	17	----------
	9	1.2 percent phenate at 90° F.; then wax emulsion	1	2	5	20	----------
	10	1.2 percent phenate in wax emulsion at 100° F	1	1	2	13	----------

A 2.0-percent solution at 80° F. gave slightly better results than a 1.2-percent solution at 100°. A 1.2-percent solution gave practically

as good control at 90° as at 100°. Following the phenate treatments with immersion in a water-wax emulsion caused no injury and gave as good control as following them with washing. Including the phenate in the water-wax emulsion caused no injury and in the few times tested it gave slight y better control than any other method.

The phenate treatments after ethylene were as satisfactory as borax before ethylene during the first 2 weeks at 70° F., but they failed to give as good control in long holding.

Three different water-wax emulsions were used in the experiments; two of them had a relatively high percentage of carnauba wax and in one the wax was largely paraffin. As there was little difference in disease control, the results with the different waxes have been combined to give the data of table 8.

Cultures were made throughout these and other disinfection experiments to determine whether *Diplodia* and *Phomopsis* were being equally controlled. Treatment with either borax or phenate before ethylene gave far better control of *Diplodia* than of *Phomopsis*. Water applications of phenate after ethylene usually gave somewhat better control of *Diplodia* than of *Phomopsis*, but when the phenate was added to a wax emulsion there seemed to be no difference in the control of the two organisms.

PRACTICAL CONSIDERATIONS

Stem-end rot is not ordinarily the cause of heavy losses during the first week after picking, a period covering the usual shipping and wholesale market operations, but it often causes heavy losses during the second week after picking when the fruit is in the hands of the retailer or consumer. These losses are borne largely by the consumer either directly or indirectly, but shippers and growers also lose because of sales resistance to their product.

The extent of this loss from decay is modified greatly by packinghouse treatment. Ethylene-treated fruit is much more likely to decay than nontreated fruit, and much can be accomplished in the control of decay by not using the treatment on fruit that does not have enough green in the rind to be definitely objectionable. For further control at least two disinfectants, borax and sodium orthophenylphenate, are available.

For ethylene-treated fruit borax is the most effective disinfectant provided the application is made before the ethylene treatment and the borax is left on the fruit during the treatment. Pre-ethylene treatment has the disadvantage of requiring an extra operation.

As a treatment after ethylene or on fruit that has not received ethylene sodium ortho-phenylphenate has been found effective, at least up to the fourteenth day after picking, with fruit held at 70° F. With the usual temperatures in transit and on the market, this period of protection would be considerably extended. Careful control of temperature and of concentration is required to prevent red specking of the rind.

SUMMARY

Methods are reported for obtaining a continuous supply of *Phomopsis* spores and of one- and two-celled *Diplodia* spores in culture.

Evidence is presented showing that the stem-end rot fungi can gain ready entrance to the fruit either through the cut on the stem or through other parts of the button.

Diplodia was found to produce decay in less than half the time required by *Phomopsis*, even at temperatures somewhat more favorable to *Phomopsis*.

Oranges that were held in an ethylene room for 42 to 45 hours at a temperature of 80° to 85° F. and a relative humidity of 87 to 92 percent were compared with similar oranges that were held for the same period in a basement at a temperature of approximately 70° and a relative humidity of 80 to 90 percent. At the end of 2 weeks after harvest the ethylene-treated fruit had about 9 times as much stem-end rot as the untreated fruit and at the end of 3 weeks more than 3 times as much. This increase was due almost entirely to *Diplodia*.

Three possible reasons are suggested for the increased decay after ethylene treatment: The higher temperatures of the ethylene room, the aging and weakening of the buttons, and a possible stimulation of the germination of *Diplodia* spores. Of these the effect upon the buttons is considered the most important.

Formaldehyde, calcium hypochlorite, sodium hypochlorite, and sodium bisulfite failed to show promise as disinfectants for the prevention of stem-end rot. Of various disinfectants tested borax and sodium ortho-phenylphenate gave the best results.

Applications of borax made after a 42- to 45-hour ethylene treatment were less than half as effective as applications made before the ethylene treatment.

Applications of sodium ortho-phenylphenate after ethylene were fully as effective as those made before ethylene.

The phenate treatments after ethylene were as effective as borax treatments before ethylene during the first 2 weeks in 70° F. storage but failed to give as good control of stem-end rot during long holding.

The phenate was used as a 1.2-percent solution at 100° F. and caused no injury when followed by thorough washing, but increases in temperature resulted in injury with this strength of solution. Nearly as good decay control was obtained at 90° as at 100° with a 1.2-percent solution, indicating that in practical operations satisfactory results should be obtained by holding the temperature in the nineties. Increasing the temperature with a particular strength of phenate solution resulted in a greater increase in injury than in decay control.

Including 1.2-percent of phenate in the water phase of a water-wax emulsion at 100° F. caused no injury, and in the few times tested gave better control of stem-end rot than any other method in which this material was used.

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A STUDY OF THE SWEET-AND-SOUR APPLE CHIMERA AND ITS CLONAL SIGNIFICANCE[1]

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INTRODUCTION

The Sweet-and-Sour apple, a form characterized by having some sour and some sweet tissue, has been known to pomologists for well over a century and a half. A contributor (*4*)[3] to the Rural New Yorker in 1897 states that 50 years earlier, in 1847, he picked 12 barrels of fruit from such a tree in an orchard near Barton Center, N. Y., where it was known as the Compound apple. E[liott] (*8*) reports the variety as having been cataloged by nurserymen as early as 1794. Numerous references to an apple variety of this description are found in the literature between 1840 and 1900, though there is no indication that it was valued or grown as anything other than a curiosity.

ORIGIN AND HISTORY

Evidently the Sweet-and-Sour apple is a variety, or type, of multiple origin, for, though cataloged at different times by a number of nurseries, it is not at all clear that they referred to the same variety. Many, if not most, of the brief descriptions imply that the single tree to which reference was being made had just appeared spontaneously, or traced back to such a single tree in some older orchard. Thus there is the record of a single tree in the orchard of Wm. T. Hamilton, Clifton Park, N. Y., though this tree was grown from scions set about 1805 (*20*). A. S. Moss (*17*), of Fredonia, N. Y., in 1855 reported having a tree bearing sweet-and-sour fruits; it is described as a seedling on which "Greening" scions had been set. Barry (*3*) reports growing such a variety for a number of years, propagated from a limb sport on a Greening tree which had been under his observation for 30 years. Besides this particular sweet-and-sour limb sport which he propagated, he reported knowing of several others.

A limb sport of this same type on an old Rhode Island Greening tree in an orchard near Breedsville, Mich., was brought to the writer's attention in 1927 and propagated by him. In 1932 scions were obtained from a similar limb sport in the orchard of D. D. Gordon, of Rushford, N. Y. This Rushford, N. Y., tree was propagated as Rhode Island Greening Selection No. 573, and its progeny furnished most of the material on which this report is based.

However, sweet-and-sour apples have not all been sports from Rhode Island Greening or from other varieties of the Greening type.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 394.

L'Hermès (*14*) has reported such a combination of red acid and yellow sweet types that was known to have originated as a seedling. Gaudichaud (*10*) has reported a similar combination of a reinette rousse and a reinette canada jaunâtre and Castle (*6*) one showing the characteristics of Boston Stripe and Golden Russet.

That many of these sweet-and-sour apples originated as limb sports there can be no doubt. Presumably most, if not all, originated either as limb or as seedling sports, though many of the early accounts describing them or telling about their occurrence suggest that some may have originated as graft hybrids developing at the point of union between stock and scion where a Greening scion was set on a sweet apple stock, or vice versa. It is significant, however, that in no instance is the evidence clear enough definitely to assign one of these sweet-and-sour forms to graft hybrid origin.

SWEET-AND-SOUR TREE AND FRUIT CHARACTERISTICS

The one distinctive characteristic of the Sweet-and-Sour variety, or type, is that the individual fruits are partly sweet and partly sour. Usually the sweet and sour portions have been described as consisting of alternating segments, of varying width, extending from base to apex, the sour sections being green in color and the sweet sections yellow (*4*, *18*, *1*). Some of the descriptions emphasize the fact that characteristically there is a clear line of demarcation between the two kinds of tissue (*16*) and that often the green, sour segments are raised above the yellow, sweet sections, giving rise to alternating ridges and hollows on the surface of the fruit. Thus the general concept of the Sweet-and-Sour apple has been that of a sectorial chimera. Other tissue patterns, however, have been observed. Avery (*2*, *p. 88*) records such a variation in pattern in the following words: * * * "upon the same apple there would be little spots about the size of the end of my finger that would be very sour, indeed, and usually these sour parts are green." In the sweet-and-sour combination described by Castle (*6*), and likewise in one cited by Darwin (*7*, *v. 1*, *p. 425–426*), the division lines between the sweet-and-sour tissues were at right angles with, rather than parallel to, the axis of the fruits.

Reversion to the all-sweet or all-sour condition has been frequently reported. Thus Avery (*2*, *p. 88*) reports: * * * "I have seen apples growing on the same limb, one would be sour and the other entirely sweet." Moss (*17*) states: "From half a bushel to one bushel [out of 10 to 12 bushels borne by the tree] will be sweet, not as large but nearly the color of Greenings, but more golden when ripe, which is earlier than the Greenings. These sweet apples * * * grow interspersed on different spurs on the same limbs with the Greenings." When such complete reversion takes place, it would be expected that scions taken for propagation purposes might produce some trees that would show complete reversion to the sour or the sweet type. Evidently this has occurred more or less frequently. One contributor (*11*) to the Rural New Yorker writes: "Several times I have had grafts sent me of the so-called sweet-and-sour apple, but on my trees, they all reverted to sour, ever anything else." Such was the experience of the author when he propagated the sweet-and-sour limb sport found near Breedsville, Mich., in 1927. Four trees of that strain have borne heavy crops for several years, all of the Rhode Island Greening type. A

somewhat more unusual type or pattern of reversion is described by Morse (*16*) as follows: "One year, the tree bore, to all appearances, only Rhode Island Greenings; the next year, some were part sweet and part sour, some all sweet and some all sour, and all these different kinds might be growing on the same branch * * * A year or two after, the tree bore a heavy crop of fine sweet apples; not one sour or mixed apple could be found. I never knew what the tree would produce until the crop presented itself." This rather strange performance is similar to one recorded for a russet sport on the Stark variety of apple, described by Swanson and Gardner (*19*).

MATERIALS USED IN THIS STUDY

Scions from the limb sport found near Breedsville, Mich., produced trees that yielded fruits exclusively of the green-skinned, sour-fleshed Rhode Island Greening type, thus showing complete reversion. Scions from the Rushford, N. Y., tree, on the other hand, produced trees that yielded sweet-and-sour fruits that, at least superficially, were like those described in the literature of two, three, and four generations ago. A number of nursery trees of this selection (No. 573) were grown; six were later set in the orchard. Their first fruits were borne in 1939. The crops were light and did not furnish enough material for a thorough study of the segregation, reversion, and peculiarities of distribution of sweet-and-sour tissue that were more or less evident. Rather careful examination, however, suggested that here is a chimera seemingly unique, a study of which might throw some light on the fundamental nature of the many horticultural varieties that belong in this general class or group.

OBJECTIVES

The specific objectives in undertaking the studies made in 1940–42 were: (1) To determine the pattern or patterns of distribution of tissues of different kinds—in this instance sweet and sour—in a supposedly sectorial chimera, (2) to determine the nature and range of distribution in sweetness or sourness of fruit borne by a single tree, (3) to determine the constancy of this range from year to year, and (4) to measure individual tree differences in respect to amount or degree of reversion and segregation.

In view of the fact that the apple is a pome fruit that includes a considerable variety of tissues—from pith to epidermis according to some students of morphology, only ovarian and floral envelope tissues according to others—it was thought that the study might throw new light on the depth of penetration of chimeral tissue mosaics.

METHODS OF PROCEDURE

Studies in 1940 were limited to field notes and observations on each of the apples borne by each of the six trees. Records were made of the amounts and distribution of sweet and sour tissue, as indicated by surface coloring of the fruit and by the width and size of the ridges, hollows, knobs, and depressions that showed differences in color. These observations were checked by organoleptic tests.

Similar records were made on the 1941 crops. It became evident, however, that more exact methods should be employed to determine the

distribution of the tissues of various degrees of sourness and sweetness within the apple. As a result of a number of preliminary tests it was found that a 0.4-percent aqueous solution of the sodium salt of bromcresol green gave a sharp color reaction to the various amounts of acidity present in the sweet-and-sour apples of this strain. By painting this solution on a fresh slice of fruit with the aid of a camel's-hair brush the acid portions of the tissue changed to a light yellow or orange yellow and the sweet tissues to a deep blue, within a few seconds. These color changes remained constant for a period of several hours so that they could be photographed. By promptly pressing the colored surfaces of the slices against a soft-textured sheet of paper its color record was readily transferred. The areas of sweet and sour tissue could then be measured with a planimeter and accurate quantitative records obtained. A number of fruits of the 1941 crop and all of the fruits of the 1942 crop were studied in this way.

PRESENTATION OF DATA

OBSERVATIONS ON 1940 AND 1941 CROPS

Observations were made in 1940 and 1941 on the distribution of fruits of various degrees of sweetness or sourness, on each of the several trees; the results are summarized in table 1. Though only four trees bore fruit in 1940 and five in 1941, certain individual tree differences were noted. Trees D and E showed a marked tendency to produce either all-sweet or predominantly sweet fruits; on the other hand, trees F and G showed an equally marked tendency to produce either all-sour or predominantly sour fruits

TABLE 1.—*Notes on tree differences in distribution of fruits possessing various proportions of sweet and sour tissue in 1940 and 1941*

Tree	Tree differences in fruit distribution for year—	
	1940	1941
D	18 fruits. 16 were all-yellow early ripening, *apparently* all-sweet. 2 were ribbed, with alternating green and yellow ribs and hollows, the green sections sour; later ripening by a week than the sweet fruits.	Yield ½ bushel. Nearly all fruits were early maturing, about 2 weeks ahead of Rhode Island Greening; small in size, comparable to Tolman Sweet. Nearly all appeared to be uniformly sweet, yellow, and regular in outline. A few had narrow raised ridges of greener, semi-acid tissue; however, in these the all-sweet tissue predominated.
E	9 fruits. 7 were all-yellow, early ripening, apparently all-sweet. 1 was early ripening and all-yellow and all-sweet, except for several green spots or knobs at the calyx end, these knobs being green and sour. 1 was late maturing (with Rhode Island Greening) and *apparently* all-green and all-sour.	Yield ¼ bushel. Fruits variable; those on certain branches *apparently* were small, all-yellow, and all-sweet; those on other branches were later maturing, ribbed, the ribs being green and sour and the hollows yellow and sweet, the sweet sectors generally being wider than the sour sectors.
F	34 fruits. 33 were all-yellow, early ripening, *apparently* all-sweet. 1 was later maturing and was all-yellow and all-sweet, except for a single green, sour sector extending from stem to calyx.	Yield ½ bushel. Fruits variable; on the average somewhat larger in size than the fruits of tree E and later in maturing, with wider green sour segments.
G	12 fruits. All were late maturing (with Rhode Island Greening). Most *appeared* to be symmetrical, all-green and all-sour. Several had narrow yellow, sweet sectors, but the green sour tissue in them predominated.	Yield ⅛ bushel. Fruits variable; those on certain branches *appeared* to be all-green, all-sour; those on other branches, the majority, were ribbed, with the ribs green and sour, the hollows yellow and sweet, and the ribs predominating over the hollows.
H	No fruits	Yield ½ bushel. Fruits variable; many all-green; in the others green predominated.
I	do	No fruits.

DISTRIBUTION OF SWEET AND SOUR TISSUE

Cross, longitudinal, and tangential sections were made through a number of representative fruits from each of the trees of the Sweet-and-Sour -variety. Sections were also made of Rhode Island Greening fruits to serve for comparison. The sections were stained as described under Methods of Procedure, and then photographed. A representative series of these photographs is presented in figures 1–6.

It will be noted that the stained cross sections of the acid to subacid Rhode Island Greening fruits (fig. 1) show some variation in degree of acidity, a variation that is associated with degree of maturity. For instance, of the two fruits on the right, the upper one was somewhat less mature than the lower one, but both show a comparatively uniform coloration from surface to center and from side to side. Slight differences in the intensity of the staining, associated with corresponding differences in amount of bromcresol green, account for part of the difference in the density of shading seen in the illustration.

Figures 2 and 3 show the distribution of sweet and sour tissue in median transverse sections of each of four representative fruits from two trees of the Sweet-and-Sour apple. They serve to emphasize the point brought out in table 1 that no two trees of this variety are alike in respect to amounts or distribution of sweet and sour tissues, except those that have more or less completely reverted to an all-sweet or an all-sour type. Indeed, they show that no two fruits on the same tree are exactly alike. When taken along with figures 4, 5, and 6, showing cross sections at different levels from the same fruit, and also radial and tangential sections, they indicate an extreme irregularity in the distribution of the two kinds of tissue within the fruit—a distribution that is more properly described as being without pattern than as having pattern. There are segments or radial sectors of sweet tissue alternating with similar segments or radial sectors of sour tissue; there are also surface patches or masses of the one subtended by larger masses of the other; there are similar islands of the one type embedded in larger masses of the other; the calyx end may be like or unlike the stem end of the same fruit; there is almost as much lack of radial as of bilateral symmetry. Certainly the distribution is not such as corresponds to current concepts of sectorial, pereclinal, or even mericlinal chimeras.

Another characteristic, perhaps suggested but not definitely established by surface color patterns and organoleptic tests, clearly brought out by these illustrations, is that for the most part the boundary lines between areas or masses of sweet and sour tissue are not sharp. Seldom can an exact line be drawn between them. Instead there is a zone, sometimes narrow, sometimes wide, where there is a mixture of the two kinds of tissue. Sometimes these areas of mixed tissue are principally of the one kind or of the other; sometimes the mixture consists of about equal parts of the two components. In some instances the mixture constitutes a relatively large part, in others a small part, of the volume or mass of the fruit.

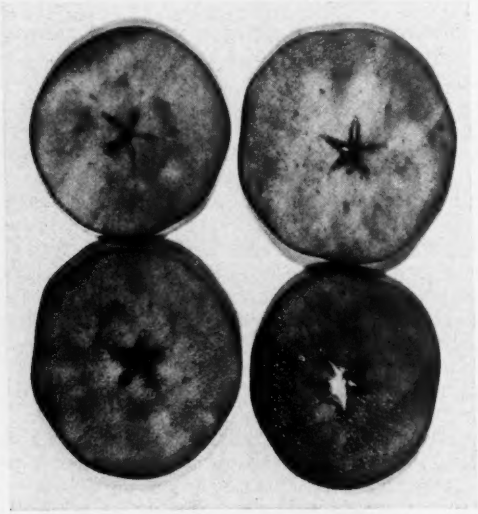

FIGURE 1.—Cross sections through the centers of four average Rhode Island Greening fruits, characterized by sour, or at least subacid, tissue, treated with a bromcresol-green solution, which stains acid tissue yellow and sweet tissue blue. Note the uniform acid tissue, indicated by the light color.

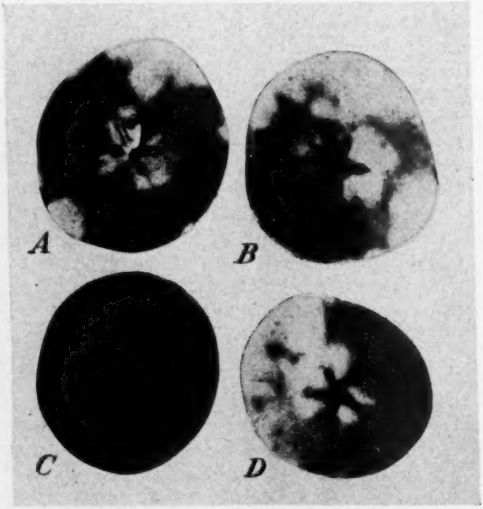

FIGURE 2.—Cross sections through the centers of four representative Sweet-and-Sour fruits from tree F, treated with a bromcresol-green solution. Note that fruit *C* is all sweet, fruit *A* more sweet than sour, and fruits *B* and *D* more sour than sweet. Also note the very irregular distribution of the two kinds of tissue.

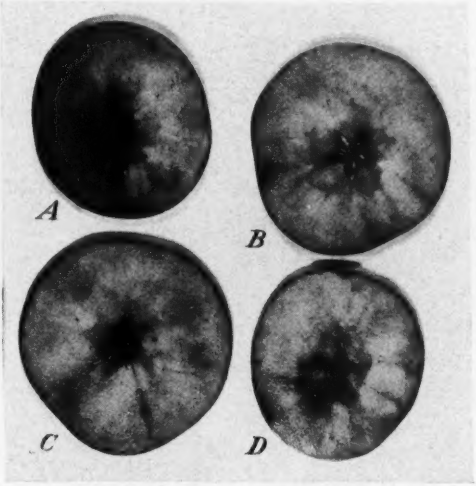

FIGURE 3.—Cross sections through the centers of four representative Sweet-and-Sour fruits from tree G, treated with a bromcresol-green solution. Note that the fruit *A* is a third to a half sweet, while *B*, *C*, and *D* are almost all-sour, though they show small radial segments or centrally located patches of subacid tissue.

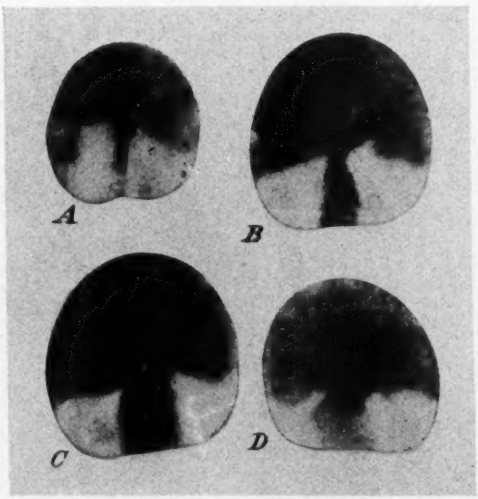

FIGURE 4.—Four cross sections, at different levels, through the same Sweet-and-Sour apple from tree D. Section *A* is from near the calyx end, *B* from just a little above its center, *C* from a little below its center, and *D* from near the stem end. Note that a larger part of the basal end of this fruit is acid than of the apical end.

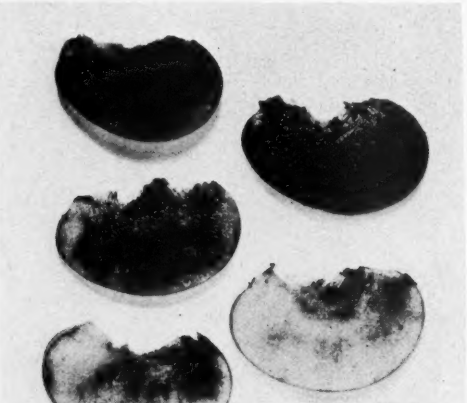

FIGURE 5.—A series of radial sections through the same apple from tree F, treated with a bromcresol-green solution. Note that, while five of the sections show principally sweet tissue and two of them principally sour tissue, the pattern of distribution of the two kinds of tissue is very irregular.

FIGURE 6.—Four vertical tangential sections through a single Sweet-and-Sour apple treated with a bromcresol-green solution. Note the very marked differences between the two sides of the apple.

QUANTITATIVE STUDIES OF THE SWEET AND SOUR TISSUES

The 1942 season crops borne by the six trees of Sweet-and-Sour Selection No. 573 were large enough so that quantitative studies could be made of the amounts of sweet and sour tissue in the fruits from different trees and statistical tests for significance applied to the data that were obtained. Every fruit of the 1942 crop was sectioned transversely through the center, the exposed surface was promptly treated with the bromcresol-green solution, and a few seconds later a print was made showing color, and therefore sweet and sour, distribution over its entire surface. Similar sections and prints were made of the apical and basal regions of each apple. Lines were then drawn on the prints that served as division lines between areas of sour and sweet tissue. These were necessarily arbitrary. The arbitrary lines that were drawn, however, represent very closely where the one or the other kind of tissue dominated over the other. Sweet and sour areas of these cross sections were then measured with a planimeter. Representative data obtained from these planimeter measurements are presented in table 2 and a summary of all the data obtained in table 3.

TABLE 2.—*Percentages of sour tissue in a representative sample of individual fruits from tree D, as indicated by planimeter measurements of sour areas in basal, median, and apical cross sections; 1942 season*

Basal section			Median section			Apical section			Average sour area
Total area	Sour area		Total area	Sour area		Total area	Sour area		
Sq. in.	*Sq. in.*	*Pct.*	*Sq. in.*	*Sq. in.*	*Pct.*	*Sq. in.*	*Sq. in.*	*Pct.*	*Pct.*
4.60	0	0	5.70	0	0	4.50	0.15	0.3	0.1
5.40	0	0	6.20	0.65	10	5.30	.45	8.5	6.2
4.60	0.50	11	5.10	.85	17	4.05	1.80	44	24
4.90	.30	6	6.10	.15	2	4.75	0	0	2.7
4.50	.10	2	5.40	.70	13	4.10	.45	11	8.7
5.20	1.30	25	5.75	2.45	43	4.60	2.50	54	40.7
5.70	0	0	6.35	.30	4.8	5.30	.40	7.6	4.1
5.30	1.70	32	6.25	2.40	38	5.10	2.85	56	42
3.50	.15	4	3.90	.55	14	3.40	.25	7	8.4
4.40	1.30	29	5.30	1.20	23	3.70	.95	26	26
5.50	.40	7	6.35	.15	2.4	5.35	0	0	3.1
Average		10.5			15.2			19.4	15.0

TABLE 3.—*Percentages of sour tissue in the fruits from the six different trees, as indicated by planimeter measurements of sour areas in basal, median, and apical cross sections; 1942 season*

Tree	Fruits	Sour tissue in—						Average sour area
		Basal section		Median section		Apical section		
		Range	Average	Range	Average	Range	Average	
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
D	73	0–97	21	0–95	22	0–79	23	22
E	26	0–47	22	0–100	19	0–100	22	21
F	144	0–100	20	0–100	16	0–100	22	19
G	29	0–100	98	7–100	97	59–100	98	98
H	99	0–100	30	0–100	35	0–100	34	33
I	4	4–100	70	14–100	52	6–100	58	60

In general, the records substantiate the observations that had been made in 1940 and 1941, some of which are summarized in table 1 and others of which are presented graphically in figures 2–6. They clearly establish the marked differences between different trees of this strain in the relative amounts of sweet and sour tissue in their fruits, the extreme variation among different fruits on the same tree, and the great variations in distribution of sweet and sour tissue within the fruit itself. On the other hand, they do not bear out the impression that was gained from a more superficial examination of the fruits and from organoleptic tests that there are many fruits that show complete reversion or segregation to the sweet or to the sour condition. As a matter of fact only 4 fruits, out of the 375 borne in 1942, showed no trace of sour tissue in any of their cross sections; only 34 fruits (approximately 9 percent) showed no trace of sweet tissue in any of their cross sections. Had a larger number of sections been made of these fruits, it is possible that small islands of tissue of the other type would have been discovered, and the number of fruits classified as segregates correspondingly reduced.

DISCUSSION

Though most of the accounts of the Sweet-and-Sour apple appearing in the literature, as well as casual observations and organoleptic tests of the fruits, suggest that the variety or type should be classified as a sectorial chimera, the data obtained from this study indicate clearly that this is not such a type. Extremely few, if any, of the fruits of the 1942 crop, studied by the staining method described, even closely approximate sectorial chimeras in their pattern of arrangement and distribution of the two kinds of tissue that are present. Nor do they correspond to Baur's concept (*5*) of such periclinal chimeras as *Cytisus adami*, obtained as a graft hybrid between *Cytisus purpureus* and *Laburnum vulgare*, or the *Solanum lycopersicum* × *S. nigrum* graft hybrid of Winkler, though in their partial and complete segregation or reversion to the component parts they more or less resemble them. They more closely resemble the concept of incomplete periclinal or mericlinal chimeras of Jørgensen and Crane (*12*) which accounts for more or less superficial patches or islands of tissue of one kind surrounded by or embedded in tissue of another kind, or perhaps the "mixed or mosaic" chimeral condition of the Golden Buckeye orange and certain other citrus fruits described by Frost (*9*). The latter interpretation is more tenable if one accepts the "appendicular" concept of the morphology of the apple fruit set forth in some detail by MacDaniels (*15*) and concurred in by many others—viz, that the fleshy tissues of the pome fruit are carpellary, being foliar in origin. According to the concept interior core tissue can be interpreted as the exterior layers of much of the fleshy part of an "inverted" xylem-phloem arrangement. As a matter of fact the data presented here lend support to this interpretation of pome tissue origin and arrangement. On the other hand, if one accepts the concept of Kraus (*13*) and many others that the core of the fruit is stelar or toral in origin and is in reality pith tissue, our ideas as to the superficial character of chimeras should be modified, for clearly both sweet and sour tissues are found to

weave many diverse patterns in the core portion of the Sweet-and-Sour apple (see especially figs. 2–4). Whichever the interpretation, the Sweet-and-Sour apple illustrates the fact that the intimate association of two kinds of tissue in the same plant organ may give rise to tissue patterns extending radially to the very center of morphologically complex parts that are as varied as the superficial color patterns of any variegated plant.

It has been pointed out that the name Sweet-and-Sour has been applied to a number of different forms of different origin. Consequently, as it stands today, Sweet-and-Sour is a type, rather than a variety, name. However, when any one of these forms is propagated vegetatively, as a number have been, the resulting group of plants is to be classed as a clonal variety. In the case of this particular variety (No. 573) and apparently in a number of the others of the same type and designated by the same name, a greater diversity is encountered than is associated with the general concept of a clonal variety. It is greater than has commonly been associated with variegated or other chimeral forms. Individual tree differences within the variety were found to be much greater than those between many more or less similar varieties—e. g., varieties belonging to the Greening or the Russet groups. Perhaps many of the variegated and chimeral forms that are now being grown would be found to have an all-pervading mixture of the genetically (?) distinct component parts, were a technique available to differentiate between them comparable to the one employed in this study.

How well or faithfully such individual tree differences can be perpetuated by special care in the selection of wood or buds for propagation cannot be predicted from this study. If, as there is accumulating evidence to indicate, a much larger proportion of our clonal varieties than have heretofore been recognized as such, are in reality chimeras, the matter becomes one of practical importance rather than just academic interest.

SUMMARY

The general characteristics of the Sweet-and-Sour apple are described as they are reported in the literature of the past 150 years and as they appeared on trees of this type in an experimental orchard.

Clear differentiation between sweet and sour tissue in the same fruit was made possible by treating an exposed section to a 0.4-percent aqueous bromcresol-green solution.

All of the trees proved to be chimeras, producing partly sweet and partly sour fruits.

Some of the trees produced fruits in which the sweet tissue predominated over the sour; other trees produced fruits in which the sour tissue predominated over the sweet. These tree differences remained constant from year to year.

Cross sections of the same fruits at different levels and also tangential and radial sections of the same fruit show extreme diversity in the sweet and sour tissue pattern or distribution.

If the Sweet-and-Sour apple is classified as a mericlinal or mosaic type of chimera, this concept implies that the pome fruit is essentially "appendicular" in its morphology.

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